FUNGAL CONTAMINATION OF DRINKING WATER

R. Russell M. Paterson
Nelson Lima
Micoteca da Universidade do Minho
Centro de Engenharia Biológica
Campus de Gualtar, Braga, Portugal

INTRODUCTION

Fungi are a ubiquitous and diverse group of unique organisms accepted as a fifth kingdom. It has been estimated that 1.5 million species exist worldwide. They range in size from massive underground structures to microscopic single-celled yeasts. A large and familiar group grow by extending filaments to absorb nutrients and use sporing structure called conidiophores for dispersal: The spores are referred to as conidia. They are observed commonly on decaying foodstuff on which some fungi produce toxins (mycotoxins). In addition, they are used to produce commercial products such as antibiotics (e.g., penicillin), organic acids (e.g., citric acid), industrial alcohol (e.g., biofuel) and enzymes (e.g., amylases). A diverse range of important foodstuffs employ fungi such as bread, beer, cheese, meats, and soy sauce. Many are plant pathogens, although their predominant role is in recycling organic matter in the environment. Increasingly they are recognized as human pathogens, particularly of immunocompromised people. Their significance in drinking water is underestimated. The range of problems for which fungi are responsible is organoleptic, infections, allergic reactions, and toxin production. Another reason fungi have not been more fully examined is that there is no rapidly affecting poisonings of humans (or other animals) associated with them in water. However, mycotoxins in foodstuffs have killed animals, including humans, although the predominant effects are probably chronic in nature.

There are two sources of fungi and products in drinking water—natural and unnatural.

NATURAL CONTAMINATION

Fungi and Products in Water

Fungi

General. This concept is self-explanatory and represents the fungal equivalent of bacterial contamination. Predominately filamentous fungi are dealt with in this entry as they have greater potential significance in water. However, the issues are complex. Kelley et al. (1) is the most up-to-date and comprehensive report of fungi in distribution systems, although (2) is an important addition. However, contamination from unnatural sources was not considered (see later).

The knowledge base concerning the occurrence of fungi in drinking waters is low. Fungi are eukaryotic organisms that grow by heterotrophic absorption of nutrients. The heterotrophy implies inclusion in heterotrophic plate counts of water, although this does not occur routinely. They are present in the environment in large amounts. However, confirmed reports of fungal contamination of drinking water are only sporadic. Fungal contamination of water has been reported for decades, although investigations have been inadequate compared with those of bacteria. Almost without exception, identifications have been to the genus rather than species level: This is not problematic in itself as species delineation can be misleading because of a less robust identification and classification system (3) compared with bacteria.

The organoleptic problems that may develop with water are not associated conventionally with fungi. Fungi can grow in waters to the extent that they are visible, and produce the same volatile compounds as the actinomycetes. These eukaryotic organisms will reduce the disinfecting power of chemicals, although not necessarily to the extent to make them ineffective. Water treatment plants will be affected (e.g., fungi will contribute to the biological load of sand filters and perhaps make them less effective). A special case is stored water where fungi can accumulate and toxin concentrations could be high within the fungal cells and/or in the water. For example, bottled water can be considered as stored.

Particular fungal groups are considered to predominate "naturally" in water in general such as the oomycetes (not strictly fungi) and hyphomycetes. Although this needs to be viewed with caution, as common fungi, such as the penicillia, aspergilli, or fusaria, are likely to present in significant numbers. The oomycetes and hyphomycetes are not associated with the problems described in this entry, except perhaps as occasional observable growth. In contrast, fungi in air and soil can reach high numbers and may enter water distributions from various locations, and vice versa. Furthermore, fungi can become established in many ways such as in sediments, plant debris, biofilms, etc.

In terms of assessing the contribution of fungi to problems in water, an overarching concern is quantification, which may have contributed to them being almost overlooked in the past. Fungi are assessed often by quoting total fungal counts on agar using a single method irrespective of the taxa that may be present. If a fungus does not spore, or is at a stage in the lifecycle when conidia do not form, they cannot be isolated in a representative manner, and conversely, neither can sporing ones. Also, what is a single fungus? Is it one conidium (which could contain more than one cell), one conidiophore, or one hyphal cell? In effect, it is impossible to record accurately what the level of fungi is in a given situation. Quantification of single-celled bacteria appears comparatively straightforward, in this respect at least. So treatments may not

Water Encyclopedia, Edited by Jay Lehr, Jack Keeley, Janet Lehr, and Thomas B. Kingery III
ISBN 0-471-44164-3

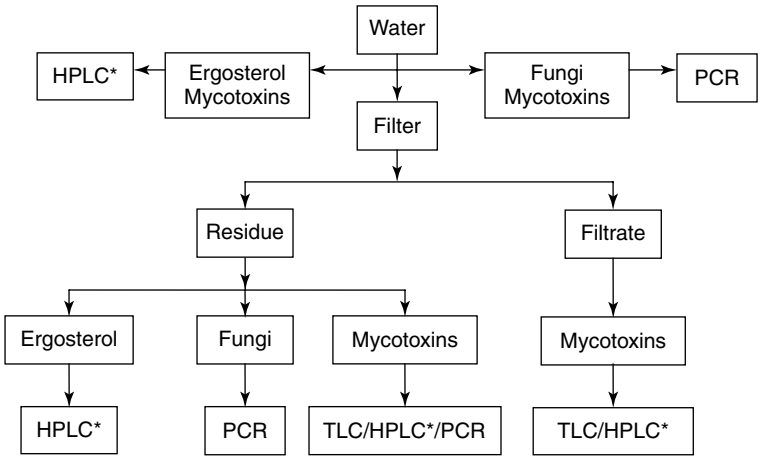

Figure 1. Flow diagram of the biochemical analysis of water for fungi and fungal metabolites. The use of commercial immunoaffinity columns is recommended for chromatographic mycotoxin analysis before or after filtration. *Developments in NMR and mass spectroscopy mean that very low levels of metabolites will be able to be identified. HPLC, high-performance liquid chromatography; TLC, thin layer chromatography; PCR, polymerase chain reaction.

reduce the total fungi but may only treat the isolation of particular growth forms. The analysis of ergosterol is now the method of choice for quantification of total biomass. It is a near universal lipid of fungi that increases in concentration with growth. Ergosterol was detected readily in water and was correlated with visual growth (4). However, the problem of taking representative sample remains where distribution of fungi is uneven.

Furthermore, fungal identifications are subjective and place too much emphasis on colony morphology minutia, which may vary in any case (3). Species designation does not imply possession of a property such as mycotoxin production, allergic response, odor, or taste. A wide range of specific gene markers and probes are now available that will, in time, be of use in drinking water (5). These may avoid problems with fungal isolations and identifications in future surveys, although whether they have been tested adequately remains to be seen.

Human Mycoses. A dramatic increase in the numbers of invasive diseases caused by filamentous fungi has occurred recently (6), which can be attributed to the large numbers of patients undergoing immunosuppresive therapies, chemotherapy, and organ and bone marrow transplants: AIDS patients are also at risk. *Aspergillus* is by far the most common and, among these, *A. fumigatus* accounts for 90% of cases. *Fusarium* and zygomycetes are common problems amongst the remainder. Pathogenic yeasts such as *Candida albicans* and *Cryptococcus neofarmans* remain very problematic. It is, of course, likely that at least some of these will be present in water supplies at some stage and, consequently, pose a threat.

Allergic Responses. In the United States, a total of 236 unique fungal allergic extract products representing 45 genera and 73 species of fungi exist (7). All are available to attempt to identify which fungi produce allergies, which indicates the potential of drinking water to be a carrier of such fungi. Furthermore, a significant number of these fungi have been reported from water.

Secondary Metabolites (Mycotoxins, Pigments, Tastes, Odors). Secondary metabolites from fungi are those that are not involved directly with the universal biochemical processes, such as DNA replication, protein formation, glycolysis, etc. These are the fungal chemicals that cause problems in water, i.e., toxins, tastes, and odors. It is likely that concentrations will be low under normal circumstances.

Mycotoxins. There are potentially thousands of these compounds (8), although approximately 10 are recognized as causing significant problems in food, feedstuff, and drink. Mycotoxins contaminate food and drink with severe effects on human/animal health, including death, cancer, immunological effects, and "disproportionately" serious ones on babies. The economic costs resulting from mycotoxins in the United States alone exceed $\$1.4 \times 10^9$ per annum. Previously unreported mycotoxins may become problematic and existing ones have effects previously undescribed (e.g., ochratoxin A and cancer). Different mycotoxins and fungi may be detected in commodities that are not considered conventionally from this perspective (e.g., water). Mycotoxins can assume increased importance by having regulations assigned to them when they were none before (e.g., recent European Union regulations on patulin).

The most important mycotoxin producers are members of the penicillia, aspergilli, fusaria, and *Claviceps* sp. Mycotoxins are considered to have directly killed people and domesticated animals in a number of specific instances at least. They also are responsible for chronic effects, such as cancers, and immunological illnesses, which are perhaps even more important: Foetuses and babies are highly susceptible. The effects of fungi on animal drinking water require consideration. It is likely to be of a poorer quality than human drinking water, which will affect animal productivity and mycotoxins in animals, which may be consumed by humans (i.e., "secondary mycotoxicosis").

Gene probes are becoming available for the detection of specific mycotoxin metabolic pathways in fungi (9–11) or environmental samples (12; 13). The novel use of a probe for the isoepoxydon dehydrogenase (IDH) gene of the patulin biosynthetic pathway has demonstrated the potential for patulin production. Consequently, it may be useful in this commodity system for identifying areas

containing patulin-producing fungi that could be subjected to control via an HACCP approach (13).

Large volumes of water are used in the manufacture of foods, feeds, and drinks. Mycotoxins could be included in these commodities, especially if this water is stored (see below). In addition, the water will often be evaporated off partially or fully during these processes, hence concentrating any mycotoxins present in the water or causing them to crystallize out of solution.

The *in vitro* studies of Kelley et al. (1) indicted that fungi could produce mycotoxins and other secondary metabolites in water. For example, zearaleneone from water inoculated with *F. graminearum* was detected; this compound has oestrogenic properties. Other mycotoxins associated with fungus were detected. Metabolites produced by other fungi were also observed and (1) should be referred to for further details. The conclusion was that mycotoxins could be produced in water.

Tastes and Odors. Many of the compounds produced by bacteria in relation to taste and odor are also produced by fungi. In addition, they produce their own battery of compounds with distinctive off-odors and tastes. Fungal isolates were capable of transforming 2,4,6-tricholorophenol to 2,4,6-trichloroanisol, which causes taste and odor problems in the distribution system. Canhoto and Magan (14) have suggested "electronic nose" technology to detect off-odors from fungi in potable water. Furthermore, a musty smell in the Parisian water system was attributed to fungi (15). A survey indicated the occurrence of taste and odor problems that were attributed to actinomycetes or algae but not fungi, for which the reasons were not obvious (1).

Pigments. Little information exists of the production of pigments from fungi in water, although they are likely to be responsible for the occurrence in some cases: Fungi, in general, produce a great deal of pigmentation. However, *in vitro* investigations indicated a red pigment from *F. graminearum* in waters (1).

Examples

Water Distribution Systems

Fungi. Systematic study of the fungi in drinking water distribution systems is lacking generally, and is usually undertaken in response to an isolated contamination problem. Furthermore, analysis for fungi using conventional techniques are time-consuming (a minimum of two weeks are required for growth and identifications), meaning that contamination problems will persist for that period of time before remedial action is taken. Various fungal isolation methods have been used in different studies making direct comparisons impossible. There have been only a few reports from distribution systems (*ca.* 15) from approximately 10 countries, and virtually none from developing countries (1). In an EU project to control the mycological quality of tap water, *Penicillium* followed by *Rhizopus* and *Cladosporium* in frequency were isolated in Portugal (unpublished results). *Exophilia* yeasts were observed. The other potential mycotoxin producers, *Alternaria* and *Chaetomium*, were isolated at low frequency.

Unsurprisingly, *Penicillium* has been shown to be common in previous studies, and no *Claviceps* were isolated (1). However, it is moderately unexpected that *Aspergillus*, especially, and *Fusarium* were not more common in water systems. A rather wide range of other genera were identified, perhaps reflecting the variety of methods used. However, many taxa were isolated from the US distribution systems examined in (1) where methods were more standardized. Unusually, many fungi were identified to the species level. The fusaria were well represented on this occasion, and the aspergilli less so. Many of the fungi were potential toxin producers. Strains were assessed for variation in nucleic acids to determine if the similar strains of species were detected throughout the systems, but natural variation within the general population was unexamined making interpretation difficult.

A 12-month survey was performed in Germany on drinking water from 29 water supplies to assess the dissemination of "hygienically" relevant fungi (2). Importantly, a correlation with standard hygiene bacterial indicators was not found. Common allergenic *Aspergillus* were rarely encountered and *Acremonium, Exophiala, Penicillium* and *Phialophora* dominated. Some were encountered throughout the drinking water system and constituted a residual flora; it was interesting that nucleic acid analysis for strain variation was considered unnecessary. In addition, soft deposits in pipelines were key sites for fungi (16).

Control of Fungi in Water Systems. Water utilities appear to have little expertise with these organisms, largely because they have not been implicated directly in health or quality problems (in water at least). Information on the (a) treatment, (b) disinfectant regimes, (c) growth strategies employed by fungi, and (d) ability to produce secondary metabolites, including mycotoxins, are scarce. In the survey undertaken in (1), treatment significantly reduced the numbers of fungi, including yeasts, in the distribution system. The study was inevitably selective. However, melanized thick-walled species predominated after treatment. Fungal counts were low throughout the system, in contradiction to results obtained by other workers. Pipe surfaces appeared to contain much larger numbers, indicating that fungi contributed to biofilms. Different species were identified from samples that suffered localized taste and odor problems, and so no correlation could be made. Equally, a correlation between numbers of fungi in the system and any of the physiochemical parameters measured in the source waters or sample sites could not be obtained. Fungi were capable of attached and unattached growth in water. It is assumed that fungi scavenge nutrients from the environment to support growth in oligotrophic (very low nutrient) situations such as water. They could colonize biofilms on pipe material after a significant period of exposure, and may be secondary colonizers once biofilm has established. Nevertheless, conventional water treatment processes result in high removal of fungi from surface water and

offer an efficient barrier to prevent fungal entry into water supplies. Filtration was better than clarification for the removal of fungi. Chlorine dioxide and ozone were most effective against fungi; free chlorine and monochloramine required longer contact periods.

In conclusion, it is probable that fungi do not behave in any particular manner that requires special procedures for control under normal circumstances. Water treatment greatly reduces the number of fungi entering water supplies, although the above provisos should be considered. Fungal conidia appear to exhibit similar susceptibilities to disinfectants as do *Giardia* cysts. However, once in the distribution system, fungi can colonize biofilms, which cannot be effectively removed by most cleaning methods currently available.

Mycotoxins. Only trace amounts of aflatoxins were detected in domestic drinking water in an analysis reported in (1). Other fungal mycotoxins and metabolites were reported in solution from water *in vitro*: Secondary metabolites from fungi isolated from water were also reported.

Stored Water

Bottled Water. Bottled water has sales of $\$5.7 \times 10^9$ and are projected to increase. Fungal contamination of bottled water is known to occur (17; unpublished results). The mycotoxin citrinin has been shown to be capable of being produced in bottled water (18) although experimental controls appeared to be inadequate in this study. It can be demonstrated as visible clumps of growth inside the bottle, which is unacceptable to the consumer, resulting in significant financial loss due to product recall and "downtime" from special sanitation treatments. However, the major loss may be the bad publicity created by such incidents.

Standardized methods to detect fungi are not currently employed by the bottling companies' quality control system. Furthermore, analysis for fungi using conventional techniques are time-consuming (a minimum of two weeks are required for growth and identifications), meaning that contamination problems will persist for that period of time before remedial action is taken. However, little information exists in the area. Bottled water is often expected to be purer compared with tap water, although this is not necessarily the case. There are anecdotal reports of bottled water being returned to the manufacture because of visual fungal growth.

Stored Water Tanks. Aflatoxins are the most significant mycotoxins, and were reported from stored water in the first reported natural occurrence of any mycotoxin (or fungal secondary metabolite) from water (19). It is also these compounds that are considered to be the most probable threat in terms of security of the water supply (see below). Zearalenone or similar compounds were also detected in the stored water. People may drink water from storage tanks whether the water is intended for that purpose or not. In the United Kingdom water is often stored in tanks for bathroom or potable purposes. Obviously, potable water is stored in certain specific situations, such cruise ships. Finally, bottled water is a form of storage.

Agricultural Runoff. It is conceivable that liquid runoff from contaminated crops or agricultural waste products could contain mycotoxins and fungi that could subsequently enter the water system.

UNNATURAL CONTAMINATION

Weapons

"Security is one of the top priorities of large and small water utilities across the USA" (20). Fungi may be considered as biological weapons. However, it is presumably their potential to produce toxins (which can be purified) that is relevant, rather than other attributes. Aflatoxins are an accepted threat, although the induction of cancers in the long-term is the predominate threat from these mycotoxins. Fungi and, particularly, mycotoxins as weapons require being more thoroughly assessed (8). However, there is no obvious fungal equivalent to the immediate response botulinum toxin would elicit, for example. One could consider a more immediate threat from ergot alkaloids, or the LSD-like effect of toxins from the mushroom *Amanita* sp. Quantity is the issue if mycotoxins are to be considered as weapons. The fungus, or more specifically the toxin, will be at much higher concentrations than would normally occur, and indeed may be able to be measured readily. Water systems or bottled water would be obvious targets. Knowledge of fungi and mycotoxins in water is more essential than ever as a result.

Mycotoxins would generally be difficult to remove if dissolved in the water supply. Boiling or autoclaving will not remove mycotoxins, although they may reduce levels in some cases. The effect of high concentrations of mycotoxins on water purifications systems is unknown, although it is likely that they would become less efficient at least. However, not all mycotoxins are water-soluble, but could be present as particles. Biosensors (e.g., protozoa) may be appropriate for detecting mycotoxins in these circumstances, as the higher concentrations would be more likely to have an effect on the test organisms. Also, they are less specific and, theoretically, any toxicity could be detected with the same test. They are inexpensive and rapid.

Fungal Biological Control Agents

These are natural fungal pathogens of insects or plants used to control insect or plant pests/diseases. The procedures tend to be experimental and generally do not compete with conventional control methods. However, commercial preparations exist and, for example, are apparently used frequently in Cuba and some developing countries. These may be applied by spray, and so may enter the water systems in this manner. Also, infected target organisms could conceivably pollute the water supply.

METHODS

The following methods have been developed and tested by the authors.

Fungal Isolations (1)

A recurring problem with isolating fungi is ensuring that they are not overgrown by bacteria. Nevertheless, ordinary media could conceivable be used as in heterotrophic plate counts. Specialized media will usually be required, containing antibacterial compounds such as antibiotics or rose bengal (RB). The growth period for fungi (e.g., 5–7 days) is considerable longer than for bacteria.

Water Distribution Systems/Bottled Water

Total Filamentous Fungi.

1. A water sample was filtered through a 0.45 μm filter.
2. This was plated onto neopeptone-glucose rose bengal aureomycin agar (NGRBA) with 5 replicates for filamentous fungal isolation and colony counting, and
3. a 100 ml sample onto half strength cornmeal agar ($\frac{1}{2}$ CMA) with 5 replicates for filamentous fungal isolation and colony counting.

Yeast

1. 100 ml samples were filtered and plated onto yeast extract malt extract glucose agar (YME) with 5 replicates for counts.

Zoosporic Fungi (Including Oomycetes). "Baiting" methods are employed to isolate these fungi, although they are not generally considered to be such a potential problem.

1. Several 0.5 cm^2 pieces of autoclaved snake skin and grass were added to 200 ml aliquots of samples in shake flasks and incubated for a maximum of 1 week at 12°C and 22°C.
2. Fungal growth was removed aseptically and plated out onto $\frac{1}{2}$ CMA.
3. The colonies were counted on plates and individual colonies identified.

Water Pipes and Sediments. Samples were "swabbed" and plate onto the above media.

Taste and Odor. Samples were treated as above. However, it is important not to smell the cultures, as this is contrary to good health and safety practice.

Analysis of Isolated Fungi for Mycotoxins

Figure 1 demonstrates a flow diagram for (a) the determination of biomass by ergosterol and (b) mycotoxin analysis. These methods are all detailed in (21), although this book is out of print and source papers may be required. References to these are available in (22).

Thin Layer Chromatography

Extracellular Secondary Metabolites

1. Fungus was grown on yeast extract sucrose (21) agar for 7 days.
2. Agar plugs (6 mm diameter) were removed from the cultures by using a sterilized cork borer.
3. These were placed at the origin of a TLC plate and then removed after 10 s.
4. TLC plate was eluted in toluene/ethyl acetate/90% formic acid (5:4:1; v/v/v).

Intracellular Secondary Metabolites

1. Cultures were grown on Czapecks yeast extract agar (21).
2. Chloroform/methanol (2:1; v/v) was placed onto the colony end of an agar plug.
3. This was touched briefly onto the origin of a TLC plate.
4. The TLC plate was eluted in chloroform/acetic acid/propan-2-ol (85:15:20; v/v/v).
5. Spots were observed in extracellular and intracellular modes by examination under white light and UV light.

Spray reagents:

1. Extracellular: *p*-anisaldehyde
2. Intracellular: Ceric sulphate

After spraying the TLC plates, repeat the white/UV light visualizations, and compare results with database in (21) or pure standards (e.g., Sigma).

HPLC

1. Cultures were grown on YES and extracted in chloroform.
2. The chloroform was evaporated and resuspended in a small volume of chloroform.
3. An aliquot was injected into an HPLC.
4. Alkylphenone standards were used to standardize the runs and so that retention indices could be calculated. The HPLC conditions involved a reversed phase column, gradient solvent system of acetonitrile water, with UV detection at 254 nm. The retention indices of peaks obtained are compared with those in databases (see 22).

PCR Mycotoxin Potential (The Example is the Isoepoxydon Dehydrogenase Gene for Patulin (10))

1. Strains were grown on malt extract agar and inoculated into 1 ml glucose yeast extract medium in Eppendorf tubes.
2. These were grown for 3 days at *ca.* 20°C.

3. Pellet were decanted.
4. DNA extracted by standard methodology.
5. The IDH gene fragment was amplified by the PCR (10).

Various other forms of analysis are possible, of course, such as DNA of isolated strains to determine variation within particular species in an attempt to determine if they are the same or different throughout a system. However, these are of limited value unless the variation within the natural population is known, and if one isolation cluster is different, it may be a different taxon.

Biochemical Analysis of Water

No other authors have published methods for this form of analysis in water except those described in (1). The use of immunoaffinity columns is recommended for analyzing water as large volumes can be used, although the affect of large volumes of water on the integrity of the packing material must be considered and the procedure requires validation. Figure 1 demonstrates a flow diagram for (a) the determination of biomass by ergosterol and (b) mycotoxins. Also, the PCR for the presence of biochemical pathways for mycotoxins, and for identification of particular taxa, is illustrated. The diagram indicates how pigmentation could be tested. The sample could be analyzed directly or after filtration where the filtrate and the eluent can be analyzed separately. The method for the direct analysis of water by PCR is similar to that given for isolated fungi above and should be referred to.

Chromatographic Analysis

1. Direct injection: This is highly convenient as it simply involves injecting the water directly into a high-performance liquid chromatograph. HPLC Water 600. UV detector. Injection volume: 100 μl, solvent: actetonitrile/water.
2. Solvent extraction: Use liquid-liquid fractionation to concentrate the mycotoxins. Chloroform is added to the water and removed in a separating funnel. The solvent is evaporated in a rotary evaporator until it is dry. Resuspend the extract in minimal volume for injection into HPLC.
3. The retention indices in the references in (22) are used to identify the peaks.
4. Immunoaffinity columns to concentrate the target compound: Elute the water of interest through an immunoaffinity column using a peristaltic pump. Volumes of 1 to 20 litres have been used by the current authors. The column can then be treated according to the manufacturer's instructions. This process has been used for aflatoxins and zearalenone. It is noted that the procedure requires validation.

Ergosterol

1. The water was filtered.
2. The residue was refluxed in 60 ml methanol/ethanol (5:1; v/v) with 10% (w/v) potassium hydroxide for 1 h.
3. This was extract with 60 ml petroleum ether which was rotary evaporated at 40°C to dryness.
4. The residue was rinsed in 15 ml of methanol that was filtered through a 0.45 μm filter (Millipore) and this was evaporated using a sample concentrator.
5. HPLC methanol (250 μl) was added and injected 100 μl into a HPLC at 282 nm. Column was a reversed phase Nova Pak C18 (3.9 × 150 mm). The solvent was HPLC methanol and the flow rate 2 ml min^{-1}.
6. The concentration of the ergosterol standards were determined by UV spectroscopy.

PCR. Do as above in section "PCR Mycotoxin Potential (The Example is the Isoepoxydon Dehydrogenase Gene for Patulin (10))", except on an aliquot of water (Fig. 1).

As mentioned above, it is possible to undertake various forms of analysis to determine strain variation on isolated fungi (e.g., DNA with the PCR), which could conceivably be used to assess if the same strains of species are being isolated throughout a distribution system. However, the degree of variation within natural populations of species is required to be assessed thoroughly as an integral part of the analysis. Otherwise, it is impossible to say to what any observed variation, or lack of, is related.

BIBLIOGRAPHY

1. Kelley, J., Kinsey, G., Paterson, R., Brayford, D., Pitchers, R., and Rossmore, H. (2003). *Identification and Control of Fungi in Distribution Systems*. AWWA Research Foundation and American Water Works Association, Denver, CO, p. 150.
2. Göttlich, E., van der Lubbe, W., Lange, B., Fiedler, S., Melchert, I., Reifenrath, M., Flemming, H-C., and de Hoog, S. (2002). Fungal flora in groundwater-derived public drinking water. *Int. J. Hyg. Environ. Health* **205**: 269–279.
3. Paterson, R. R. M., Venâncio, A., and Lima, N. (2004). Solutions to *Penicillium* taxonomy crucial to mycotoxin research and health. *Res. in Microbiol.* **155**: 507–513.
4. Kelley, J. and Paterson, R. R. M. (2003). Comparisons of ergosterol to other methods for determinations of *Fusarium graminearum* biomass in water as a model system. In: *Biological Resource Centres and the use of Microbes, European Culture Collections' Organisation XXII–Braga, September 17–19*. N. Lima and D. Smith (Eds.). Micoteca da Universidade do Minho, Braga, pp. 235–239.
5. Haughland, R. A., Varma, M. M., Wymer, L. J., and Vesper, S. J. (2004). Quantitative PCR analysis of selected *Aspergillus, Penicillium* and *Paecilomyces* species. *Systemat. Appl. Microbiol.* **27**: 198–210.
6. Sheppard, D. C., Ibrahim, A. S., and Edwards, J. E., Jr. (2004). Human mycosis: The role of molecular biology. In: *Advances in Fungal Biotechnology for Industry, Agriculture and Medicine*. J. S. Tkacz and L. Lange (Eds.). Kluwer Academic/Plenum Publishers, New York, pp. 361–384.
7. Esch, R. E. (2004). Manufacturing and standardizing fungal allergen products. *J. Allergy Clin. Immunol.* **113**: 210–215.

8. CAST. (2003). *Mycotoxins: Risks in Plant, Animal, and Human Systems*. Council for Agricultural Science and Technology, Ames, IA, p. 217.
9. Edwards, S. G., O'Callaghan, J., and Dobson, A. D. W. (2002). PCR- based detection and quantification of mycotoxigenic fungi. *Mycologic. Res.* **106**: 1005–1025.
10. Paterson, R. R. M., Kozakiewicz, Z., Locke, T., Brayford, D., and Jones, S. C. B. (2003). Novel use of the isoepoxydon dehydrogenase gene probe of the patulin metabolic pathway and chromatography to test penicillia isolated from apple production systems for the potential to contaminate apple juice with patulin. *Food Microbiol.* **20**: 359–364.
11. Paterson, R. R. M. (2004). The isoepoxydon dehydrogenase gene of patulin biosynthesis in cultures and secondary metabolites as candidate PCR inhibitors. *Mycologic. Res.* **108**: 1431–1437.
12. Paterson, R. R. M., Archer, S., Kozakiewicz, Z., Lea, A., Locke, T., and O'Grady, E. (2000). A gene probe for the patulin metabolic pathway with potential use in novel disease control. *Biocontrol Sci. Technol.* **10**: 509–512.
13. Paterson, R. R. M. (2005 In Press). Primers from the isoepoxydon dehydrogenase gene of the patulin biosynthetic pathway to indicate critical control points for patulin contamination of apples. *Food Control*.
14. Canhoto, O. F. and Magan, N. (2003). Potential for detection of microorganisms and heavy metals in potable water using electronic nose technology. *Biosens. Bioelectron.* **18**: 751–754.
15. Monteil, A., Rigal, S., and Welte, B. (1999). Study of the origin of musty taste in the drinking water supply. *Water Sci. Technol.* **40**: 171–177.
16. Zacheus, O. M., Lehtola, M. J., Korhonen, L. K., and Martikainen, P. J. (2001). Soft deposits, the key site for microbial growth in drinking water distribution networks. *Water Res.* **35**: 1757–1756.
17. Cabra, D. and Fernández Pinto, V. E. (2002). Fungal spoilage of bottled mineral water. *Int. J. Food Microbiol.* **72**: 73–76.
18. Criado, M. V., Pinto, E. V. F., Badessari, A., and Cabral, D. (2005). Conditions that regulate the growth of moulds inoculated into bottled water. *Int. J. Food Microbiol.* **99**: 343–349.
19. Paterson, R. R. M., Kelley, J., and Gallagher, M. (1997). Natural occurrence of aflatoxins and *Aspergillus flavus* (LINK) in water. *Lett. Appl. Microbiol.* **25**: 435–436.
20. Mays, L. W. (2004). *Water Supplies Systems Security*. AWWA Products, Denver, CO, p. 429.
21. Paterson, R. R. M. and Bridge, P. D. (1994). *Biochemical Techniques for Filamentous Fungi*. IMI Techniques Series No. 1, CAB International, Wallingford, UK, p. 125.
22. Paterson, R. R. M. (2004). Standardized data on 474 fungal metabolites or "extrolite". *Mycologic. Res.* **108**: 596–597.